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Main Menu | Search Form | Posting Counts | Show S Numbers | Edit S Numbers | Preferences

Search Results -

Terms	Documents
l9 same population	3

Database: US Patents Full-Text Database; US Pre-Grant Publication Full-Text Database; JPO Abstracts Database; EPO Abstracts Database; Derwent World Patents Index; IBM Technical Disclosure Bulletins

Refine Search: l9 same population Clear

Search History

Today's Date: 4/13/2001

DB Name	Query	Hit Count	Set Name
USPT	l9 same population	3	L11
USPT	l9 same zone	1	L10
USPT	l4 same heterogeneous	79	L9
USPT	l6 same bind$	10	L8
USPT	l6 same immobiliz$	0	L7
USPT	(heterogeneous near0 population) same DNA	85	L6
USPT	l2 and l4	5	L5
USPT	DNA same immobiliz$	3793	L4
USPT	l2 same immobiliz$	0	L3
USPT	l1 same (heterogeneous near0 population)	45	L2
USPT	DNA same sequenc$	29278	L1

look at L9

L9: Entry 4 of 79 File: USPT Dec 19, 2000

DOCUMENT-IDENTIFIER: US 6162603 A
TITLE: Hybridization of polynucleotides conjugated with chromophores and fluorophores to generate donor-to-donor energy transfer system

DEPR:
Alternatively, a heterogeneous format widely used is the Southern blot procedure in which genomic DNA is electrophoresed after restriction enzyme digestion, and the electrophoresed DNA fragments are first denatured and then transferred to an insoluble matrix. In the blot procedure, a polynucleotide probe is then hybridized to the immobilized genomic nucleic acids containing complementary nucleic acid (target) sequences.

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L5: Entry 2 of 5 File: USPT Nov 17, 1998

DOCUMENT-IDENTIFIER: US 5837500 A
TITLE: Directed evolution of novel binding proteins

BSPR:
In order to obtain the display of a multitude of different though related potential binding domains, applicants generate a heterogeneous population of replicable genetic packages each of which comprises a hybrid gene including a first DNA sequence which encodes a potential binding domain for the target of interest and a second DNA sequence which encodes a display means, such as an outer surface protein native to the genetic package but not natively associated with the potential binding domain (or the parental binding domain to which it is related) which causes the genetic package to display the corresponding chimeric protein (or a processed form thereof) on its outer surface.

DEPR:
Other immobilization methods depend on the presence of particular chemical functionalities. A polypeptide will present --NH.sub.2 (N-terminal; Lysines), --COOH (C-terminal; Aspartic Acids; Glutamic Acids), --OH (Serines; Threonines; Tyrosines), and --SH (Cysteines). A polysaccharide has free --OH groups, as does DNA, which has a sugar backbone.

DEPR:
MK-BPTI and MK phage were diluted to a concentration of 1.4.multidot.10.sup.12 particles per ml in TBS buffer (PARM88) containing 1.0 mg/ml BSA. We added 4.0.multidot.10.sup.10 phage to 5 microliters of a 50% slurry of either agarose-immobilized anhydro-trypsin beads (Pierce Chemical Co.) or agarose-immobilized streptavidin beads (Sigma) in TBS/BSA. Following a 3 hour incubation at room temperature, the beads were pelleted by centrifugation for 30 seconds at 5000 rpm in a microfuge and the supernatant fraction was collected. The beads were washed 5 times with TBS/Tween buffer (PARM88) and after each wash the beads were pelleted by centrifugation and the supernatant was removed. Finally, beads were resuspended in elution buffer (0.1N HCl containing 1.0 mg/ml BSA adjusted to pH 2.2 with glycine) and following a 5 minute incubation at room temperature, the beads were pelleted by centrifugation. The supernatant was removed and neutralized by the addition of 1.0M Tris-HCl buffer, pH 8.0. Aliquots of phage samples were applied to a Nytran membrane using a Schleicher and Schuell (Keene, N.H.) filtration minifold and phage DNA was immobilized onto the Nytran by baking at 80.degree. C. for 2 hours. The baked filter was incubated at 42.degree. C. for 1 hour in pre-wash solution (MANI82) and pre-hybridization solution (5Prime-3Prime, West Chester, Pa.). The 1.0 Kb NarI (base 1630)/XmnI (base 2646) DNA fragment from MK RF was radioactively labelled with .sup.32 P-dCTP using an oligolabelling kit (Pharmacia, Piscataway, N.J.). The radioactive probe was added to the Nytran filter in hybridization solution (5Prime-3Prime) and, following overnight incubation at 42.degree. C., the filter was washed and subjected to autoradiography.

DEPR:
Aliquots of phage samples were applied to a Nytran membrane using a Schleicker and Schuell minifold apparatus. Phage DNA was immobilized onto the Nytran by baking at 80.degree. C. for 2 hours. Filters were washed for 60 minutes in pre-wash solution (MANI82) at 42.degree. C. then incubated at 42.degree. C.

for 60 minutes in Southern pre-hybridization solution (5Prime-3Prime). The 1.0 Kb NarI (1630bp)/XmnI (2646 bp) DNA fragment from MK RF was radioactively labelled with .sup.32 P-.alpha.dCTP using an oligolabelling kit (Pharmacia, Piscataway, N.J.). Nytran membranes were transferred from pre-hybridization solution to Southern hybridization solution (5Prime-3Prime) at 42.degree. C. The radioactive probe was added to the hybridization solution and following overnight incubation at 42.degree. C., the filter was washed 3 times with 2.times.SSC, 0.1% SDS at room temperature and once at 65.degree. C. in 2.times.SSC, 0.1% SDS. Nytran membranes were subjected to autoradiography. The efficiency of the affinity selection system can be semi-quantitatively determined using the above dot blot procedure. Comparison of dots A1 and B1 or C1 and D1 indicates that the majority of phage did not stick to the streptavidin-agarose beads. Washing with TBS/Tween buffer removes the majority of phage which are non-specifically associated with streptavidin beads. Exposure of the streptavidin beads to elution buffer releases bound phage only in the case of MK-BPTI phage which have previously been incubated with biotinylated rabbit anti-BPTI IgG. This data indicates that the affinity selection system described above can be utilized to select for phage displaying a specific antigen (in this case BPTI). We estimate an enrichment factor of at least 40 fold based on the calculation ##EQU2##

DEPR:
Two hundred nanograms of the purified single-stranded DNA were annealed to 3 picomoles of the phosphorylated 25 mer mutagenic oligonucleotide (P1). Following filling in with T4 DNA polymerase and ligation with T4 DNA ligase, the sample was used to transfect competent cells which were subsequently plated on LB plates: to permit the formation of plaques. Phage derived from picked plaques were applied to a Nytran membrane using a Schleicher and Schuell (Keene, N.H.) minifold I apparatus (Dot Blot Procedure). Phage DNA was immobilized onto the filter by baking at 80.degree. C. for 2 hours. The filter was bathed in 1.times.Southern pre-hybridization buffer (5Prime-3Prime, West Chester, Pa.) for 2 hours. Subsequently, the filter was incubated in 1 X Southern hybridization solution (5Prime-3Prime) containing a 21 mer probing oligonucleotide (LEU1) which had been radioactively labelled with gamma-.sup.32 P-ATP (N.E.N./DuPont, Boston, Mass.) by T4 polynucleotide kinase (New England BioLabs (NEB), Beverly, Mass.). Following overnight hybridization, the filter was washed 3 times with 6.times.SSC at room temperature and once at 60.degree. C. in 6.times.SSC prior to autoradiography. Clones exhibiting strong hybridization signals were chosen for large scale Rf preparation using the PZ523 spin column protocol (5Prime-3Prime). Restriction enzyme analysis confirmed that the structure of the Rf was correct and DNA sequencing confirmed the substitution of a LEU codon (TTG) for the LYS.sub.15 codon (AAA). This Rf DNA was designated MB46(K15L).

DEPR:
The pH 2.0 fraction from the third enrichment cycle of the MYMUT library was titered on a lawn of cells. Twenty plaques were picked at random. Rf DNA was prepared for each of the clones and fusion phage were collected by PEG precipitation. Clonally pure populations of fusion phage in TBS/BSA were prepared and characterized with respect to their affinity for immobilized HNE. pH elution profiles were obtained to determine the stringency of the conditions required to elute bound fusion phage from the HNE matrix. FIG. 9 illustrates the pH profiles obtained for EpiNE clones 1, 3, and 7. The pH profiles for all 3 clones exhibit a peak centered on pH 3.5. Unlike the pH profile obtained for the third passage of the MYMUT library, no minor peak centered on pH 4.5 is evident. This is consistent with the clonal purity of the selected EpiNE phage utilized to generate the profiles. The elution peaks are not symmetrical and a prominent trailing edge on the low pH side. In all probability, the 10 minute elution period employed is inadequate to remove bound fusion phage at the low pH conditions. EpiNE clones 1 through 8 have the following characteristics: five clones (identified as EpiNE1, EpiNE3, EpiNE5, EpiNE6, and EpiNE7) display very similar pH profiles centered on pH 3.5. The remaining 3 clones elute in the pH 3.5 to 4.0 range. There remains some diversity amongst the 20 randomly chosen clones obtained from the pH 2.0 fraction of the third passage of the MYMUT library and these clones might

exhibit different affinities for HNE.

DEPR:
In a third round of affinity selection, a population of phage particles containing 3.0.multidot.10.sup.10 plaque forming units was added to 20 .mu.l of 50% slurry of agarose-immobilized HNE beads and incubated for 2 hours at room temperature. We eluted the phage with the following pH washes: pH 7.0, 6.0, 5.0, 4.5, 4.0, 3.5, 3.25, 3.0, 2.75, 2.5, 2.25, and 2.0. After plating a portion of the pH 2.0 eluate fraction for plaque formation, we picked individual plaques for preparation of RF DNA. DNA sequencing yielded the amino acid sequence in the mutated secondary loop for 20 EpiNE7 homolog clones. These sequences, together with EpiNE7, are given in Table 210 as EpiNE7.21 through EpiNE7.40. The plaques observed when EpiNEs are plated display a variety of sizes. EpiNE7.21 through EpiNE7.30 were picked with attention to plaque size: 7.21, 7.22, and 7.23 from small plaques, 7.24 through 7.30 from plaques of increasing size, with 7.30 coming from a large plaque. TRP occurs at position 39 in EpiNE7.21, 7.22, 7.23, 7.25, and 7.30. Thus plaque size does not correlate with the appearance of TRP at 39. One sequence, EpiNE7.31, from this fractionation is identical to sequences EpiNE7.8 and EpiNE7.9 obtained in the first fractionation. EpiNE7.30, EpiNE7.34, and EpiNE7.35 are identical, indicating that the diversity of the library has been greatly reduced. It is believed that these sequences have an affinity for HNE that is at least comparable to that of EpiNE7 and probably higher. Because the parental EpiNE7 sequence did not recur, it is quite likely that some or all of the EpiNE7.nn derivatives have higher affinity for HNE than does EpiNE7.